



PO/AU00/01018

09/830438

REC'D 10 OCT 2000

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I, KAY WARD ACTING MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 2469 for a patent by AUSTRALIAN NATIVE FOODS MANAGEMENT LTD filed on 26 August 1999.

WITNESS my hand this
Twenty-fifth day of September 2000

K Ward

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ACTING MANAGER EXAMINATION
SUPPORT AND SALES



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AUSTRALIA
Patents Act 1990

Australian Native Foods Management Limited

ORIGINAL

**PROVISIONAL SPECIFICATION
STANDARD PATENT**

Invention Title:

Insecticidal Composition

The following statement is a full description of this invention
including the best method of performing it known to us:-

Technical Field

The present invention relates to an insecticidal composition including Tasmannia stipitata extract and insecticidally effective oils such as petroleum oils, vegetable oils and/or fish oils. The invention also relates to a method of controlling insects using combinations of Tasmannia stipitata extract and insecticidally effective oils such as petroleum oils, vegetable oils and/or fish oils.

Background Art

The genus Tasmannia was formerly known as Drimys. Tasmannia stipitata (also known previously as Tasmannia aromatica var. pendunculata or Drimys aromatica) is commonly known as Dorrigo Pepper. It is rich in the compound polygodial.

Polygodial is used in foods for its hot and spicy taste. It also has been found to have antifungal, antibacterial and insecticidal activity.

The active ingredients of Tasmannia stipitata appear to be contained within oil cells concentrated in new leaves, new stems and berries. Extracts of leave, stems and berries include polygodial as well as linalool, pinene, copaene, caryophyllene and bicyclo-germacrene.

Petroleum oils (or mineral oils) are used as insecticides. Certain vegetable oils also have insecticidal activity. The most common is canola oil (rapeseed oil). Sesame oil is also used. Other vegetable oils having insecticidal activity include mustard oil. A third group of oils having insecticidal activity is fish oil.

Disclosure of Invention

Combinations of Tasmannia stipitata extract and petroleum oil or insecticidally effective vegetable oil have been found to have unexpected synergistic activity as insecticides. It is assumed that the insecticidal activity of fish oils would be similarly enhanced in combination with Tasmannia stipitata extract.

Accordingly, the present invention provides an insecticidal composition including Tasmannia stipitata extract and an insecticidally effective oil selected from the group consisting of at least one petroleum oil, at least one vegetable oil, at least one fish oil and mixtures thereof.

In addition to insecticidal activity, the composition acts as an insect repellent. It has also been found that insects that come into contact with a surface that has been treated with the composition exhibit reduced feeding

and egg laying behaviour. The composition may therefore be used to control insects.

In another aspect, the present invention provides a method of controlling or killing insects comprising exposing an insect population to a composition including *Tasmannia stipitata* extract and an insecticidally effective oil selected from the group consisting of at least one petroleum oil, at least one vegetable oil, at least one fish oil and mixtures thereof.

In a further aspect, the present invention provides for the use of *Tasmannia stipitata* extract together with an insecticidally effective oil selected from the group consisting of at least one petroleum oil, at least one vegetable oil, at least one fish oil and mixtures thereof, for controlling or killing insects.

The extract of *Tasmannia stipitata* is preferably prepared by solvent extraction of new leaves, new stems and berries. The concentration of polygodial in the extract will vary with the relative proportions of leaves, stems and berries extracted and will also vary with season. Concentrations of about 10% or more polygodial are desirable in the extract, preferably about 10-40% and more preferably about 25-38%.

The concentration of *Tasmannia stipitata* extract in the insecticidal composition may range from about 0.01% w/v to about 1.25% w/v, preferably 0.02% w/v to 1.0% w/v, more preferably 0.03% w/v to 0.125% w/v. The desired concentration will vary with the polygodial content of the *Tasmannia stipitata* extract.

The concentration of insecticidally effective oil in the insecticidal composition may range from about 0.25% w/v to about 1.5% w/v, preferably 0.5% to 1.0% w/v.

Modes for Carrying Out the Invention

Materials

1. Samples of *Tasmannia stipitata* extract (hereinafter referred to as "stipitata extract") were prepared by solvent extraction of mixtures of new leaves, new stems and berries of *Tasmannia stipitata*. These samples contained an average of 27.6% polygodial. The polygodial content of the samples was ascertained as follows:

A known weight of stipitata extract was dissolved in 5 ml of ethanol with an internal standard of tridecane at a concentration of 2mg/ml. This solution was analysed by gas chromatography

and the polygodial was assessed by area ratio to the tridecane and assumption that the response factor between the two compounds equals 1. The average percent of polygodial stated above is the average percent of volatiles in the extract.

- 5 2. D-C-Tron Plus®, spray oil, supplied by Ampol, active ingredient 839g/L petroleum oil.
3. Fasta® oil, supplied by Cobbett Pty Ltd, 704 g/L vegetable oil, primarily canola oil (hereinafter referred to as "canola oil").
- 10 4. White oil spray, supplied by Yates, active ingredient 820g/L petroleum oil (hereinafter referred to as "white oil").

Process

Stipitata extract in amounts shown in column A of the table below was combined with oils B, C or D respectively in amounts as shown below.

	A	B	C	D
Mixtures	Weight of Stipitata extract (g)	Weight of D-C-Tron Plus® (g)	Weight of Canola Oil (g)	Weight of white oil (g)
1	1.25	10	10	10
2	0.625	10	10	10
3	0.3125	10	10	10
4	1.25	5	5	5

15

Each of these blends was diluted with distilled water in a 100ml volumetric flask to prepare the following mixtures: Stipitata extract(ml) and oil(ml) (1.25 and 10), (0.625 and 10), (0.3125 and 10) and (1.25 and 5) per 100ml dilution.

20

From these homogenised stock solutions, further serial dilutions to (0.125 and 1), (0.0625 and 1), (0.03125 and 1), (0.125 and 0.5), (0.0625 and 0.5) and (0.03125 and 0.5) per cent were prepared by mixing the required amount of the stock solution in distilled water.

25

For Stipitata extract alone, one gram was dissolved in 20ml of absolute ethanol and distilled water was added to prepare a 1% stock solution. A drop weighing 40mg Triton X-100® surfactant supplied by Union Carbide

Chemicals and Plastic Co, was added to the stock solution. Further serial dilutions of 0.01, 0.02, 0.04, 0.06 and 0.08% were prepared by mixing the required amount of stock solution with distilled water.

5 For each oil alone a 10% w/v emulsion in distilled water and further dilutions of 2.0, 1.8, 1.6, 1.4, 1.2 1.0, 0.50 and 0.25% were prepared by adding to the required amount of distilled water.

Adult female two-spotted mite (*Tetranychus urticae*) were collected.

10 Tests were conducted on 80 females for each treatment, distributed on four French bean leaf discs 25mm diameter, contained in 90mm diam petri dishes. Each treatment was repeated twice.

The leaf discs were placed on moist cotton wool covered with muslin netting.

Water was added to the dish daily to prevent the desiccation of the leaf discs.

15 The dose of product for testing was applied by means of a Potter Precision Spray Tower and a 5ml aliquot was used for each petri dish.

The mortality (number killed) was assessed 24, 48 and 72 hours after treatment. Death was recognised by the absence of movement when the test mites were mechanically stimulated.

20 Probit analysis (a standard method to determine toxicity of pesticides) based on the concentration of Stipitata extract in the mixtures was carried out to interpret the dose mortality relationship.

Results

Results are summarised in the tables below.

PRODUCT	TIME IN HOURS	LD95		
		LOWER	MEDIAN	HIGHER
Stipitata extract	24	0.13	0.19	0.3
	48	0.06	0.12 (a)	0.77
	72			
D-C-Tron Plus®	24	1.75	2.72 (c)	67.06
White oil	24	2.50	3.04	4.23
	48			
	72			
Canola oil	24	3.85	4.60	5.61
	48	3.41	3.71 (e)	4.47
	72			
(% D-C-Tron Plus®) + Stipitata extract	(0.5%) 24		0.196	
	(1%) 24		0.07	
	(0.5%) 48	0.061	0.048 (b)	0.085
	(1%) 48	0.03	0.04	0.06
	(0.5%) 72		0.063	
	(1%) 72		0.03	
(% Canola Oil) + Stipitata extract	(0.5%) 24		0.012	
	(1%) 24	0.037	0.068	0.325
	(0.5%) 48		0.11	
	(1%) 48		0.08 (d)	
	(0.5%) 72		0.069	
	(1%) 72		0.049	

PRODUCT	TIME IN HOURS	LD50		
		LOWER	MEDIAN	HIGHER
Stipitata extract	24	0.03	0.03	0.04
	48	0.02	0.003	0.03
	72			
D-C-Tron Plus®	24	0.44	1.23	12.85
White oil	24	1.23	1.54	1.88
	48			
	72			
Canola oil	24	1.81	2.02	2.34
	48	1.54	1.69	1.88
	72			
(% D-C-Tron Plus®) + Stipitata extract	(0.5%) 24		0.023	
	(1%) 24		0.02	
	(0.5%) 48	0.001	0.006	0.011
	1% 48	0.002	0.01	0.017
	(0.5%) 72		0.016	
	(1%) 72		0.01	
(% Canola oil) + Stipitata extract	(0.5%) 24		0.03	
	(1%) 24		0.01	
	(0.5%) 48		0.02	
	(1%) 48		0.017	
	(0.5%) 72		0.018	
	(1%) 72			0.017

Combined with all the oils outlined above, Stipitata extract was more efficacious (ie. caused higher mortality) than when used alone. In addition, for oils to which Stipitata extract had been added, efficacy was significantly enhanced. For oils used alone, mortality was lowest of all treatments.

5 The results above demonstrate synergistic insecticidal effect against Two Spotted Mite by Tasmannia stipitata extract mixed with different types of oils. The LD95 of Stipitata extract alone is improved from 0.12% (a) to 0.048% (b) when blended with 0.5% petroleum oil. Simultaneously the LD95 of petroleum oil is improved from 2.72% (c) to 0.048% (b) with the addition
10 of Stipitata extract.

The LD95 of Stipitata extract alone is improved from 0.12% (a) to 0.08% (d) when blended with 1% canola oil. Simultaneously the LD95 of canola oil is improved from 3.71% (e) to 0.08% (d) with the addition of Stipitata extract.

15 Synergistic activity as an insecticide against a broad range of insects is expected including thrips, aphids, scales, mites, caterpillars, lice and flies.

Industrial Applicability

Combining Tasmannia stipitata extract with insecticidally effective oils including petroleum oil, vegetable oil, and fish oil gives a highly effective
20 insecticidal composition. The proportion of active ingredients in the composition required for effective control of the insect pest can be substantially reduced compared to the proportion required for each ingredient when used alone. Phytotoxicity of the composition is significantly reduced by reduced actives levels, as is the cost of manufacturing the
25 composition. It is expected that the insecticidal composition will be effective against a broad range of insects.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the
30 invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this twenty sixth day of August 1999

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